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Summary

This annual report contains a listing of the task orders and work performed on each during the period of September 1, 1990 through August 31, 1991 for Contract No. DAMD17-85-C-5133. Appendix A lists the issue dates for the final reports for Task Orders # 7, 8, 9, 10, 11, 12, 13, and 14. Appendices B, C, D, F, G, H, I, and J provide summaries of the results of the Task Orders. Appendix E is a copy of the abstract of the results of Task Order #10.

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Foreword

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

For the protection of human subjects the investigators have adhered to policies of applicable Federal Law 45CFR46.

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Body of Report

During this period of the contract, our group worked on the following Task Orders:

1. Task Order #7

Title: "Pharmacokinetics and Pharmacodynamics of Sustained, Low-dose, Intravenous Infusions of Pyridostigmine"

The clinical portion was conducted between July 27, 1987 and October 16, 1987 and evaluation of the data continued. The date the final report was issued is listed in Appendix A. Appendix B is the summary of the results.

2. Task Order #8

Title: "Safety, Tolerance, Pharmacokinetics and Pharmacodynamics of Single Oral Doses of Sustained-release Pyridostigmine in Healthy Men"

The clinical portion was initiated on January 11, 1988 and was completed on April 23, 1988. The preparation of draft task reports was under way. Several drafts had been submitted by our group to the Contracting Officer Representative, Col. Brian Schuster, for his review and review by his associates. The suggestions for revisions based on those reviews were incorporated into additional draft task reports, and by the end of this reporting period the process of finalizing the task report was almost completed. The date the final report was issued is listed in Appendix A. Appendix C summarizes the results.

3. Task Order #9

Title: "Safety, Tolerance, Pharmacokinetics and Pharmacodynamics of Single Oral Doses of Sustained-release Pyridostigmine (Duphar) in Healthy Men"

The clinical portion was conducted between March 28, 1988 and April 30, 1988. Draft task reports had been prepared and revised. Several drafts had been submitted for review by Col. Schuster and his associates. The suggested revisions based on those reviews were incorporated into additional drafts, and by the end of this reporting period the process of finalizing the task report was almost completed. The date the final report was issued is listed in Appendix A. The results of the study are summarized in Appendix D.

4. Task Order #10

Title: "Multiple-dose Pharmacokinetics, Safety and Tolerance of WR 6026 Hydrochloride in Healthy Subjects"

The clinical portion of this project was initiated on August 16, 1988 and had been completed on December 16, 1988. The data were collected and analyzed for drafting the task report. The draft task report was submitted to Col. Schuster for review by him and by his associates. Suggestions for improvement were made, and substantial dialogue followed as additional draft task reports were exchanged and reviewed. In addition, in collaboration with Col. Schuster, it was decided to have all of the electrocardiograms recorded in this study reviewed independently by a cardiologist. The process of review and revision of the draft task reports was completed shortly before the end of this reporting period, with the formal Task Report being issued on August 21, 1991. An abstract describing the results of the study was submitted to the 31st Interscience Conference on Antimicrobial Agents and Chemotherapy, and was accepted for presentation at this meeting which took place in Chicago, Illinois over the period September 29-October 2, 1991 (Appendix E). The results are summarized in Appendix F.

5. Task Order #11

Title: "Safety, Tolerance, Pharmacokinetics and Pharmacodynamics of Single Oral Doses of Pyridostigmine Administered by an Osmotic-delivery Module (Osnet^R) Compared to Pyridostigmine Syrup in Healthy Men"

The clinical portion of this project, which began on January 8, 1989, had been completed on February 3, 1989. The data had been collected and analysis initiated for preparing the task report. During this reporting period, several drafts of the task report were submitted for review by Col. Schuster and his associates. The suggested revisions based on those reviews were incorporated into additional drafts, and by the end of this reporting period the process of finalizing the task report was almost completed. The date the final report was issued is listed in Appendix A. Appendix G provides a summary of the results.

6. Task Order #12

Title: "Safety, Tolerance, Pharmacokinetics and Pharmacodynamics of Single Oral Doses of a Commercial Formulation of Sustained-release Pyridostigmine in Healthy Men"

The clinical portion of this project was conducted between December 12 and December 23, 1988. The data had been collected and analysis initiated for preparing the task report. During this reporting period, several drafts of the task report were submitted for review by Col. Schuster and his associates. The suggested revisions based on those reviews were incorporated into additional drafts, and by the end of this reporting period the process of finalizing the task report was almost completed. The date the final report was issued is listed in Appendix A. The results of this Task Order are summarized in Appendix H.

7. Task Order #13

Title: "A Protocol to Assess the Irritancy, Contact Sensitization and Contact Photoallergic Potential of Niclosamide - A Topical Anti-schistosomal Agent"

The clinical portion of this study was initiated on December 21, 1989 and was completed on July 20, 1990. Throughout the conduct of this study, close collaboration continued between our group and Army personnel at Walter Reed and at Ft. Detrick. Necessary amendments to the protocol were made to facilitate the completion of the project in a proper fashion. The data were analyzed and drafts of the task report were prepared and submitted for review by Col. Schuster and other Army personnel. Using the suggestions coming from these reviews, and after discussion within our group, the drafts were revised and reviewed again. The final report was issued on April 18, 1991. Appendix I summarizes the results.

8. Task Order #14

Title: "Safety, Tolerance, Pharmacokinetics and Pharmacodynamics of Intravenous Pyridostigmine and Oral Doses of Standard and Sustained-release Pyridostigmine in Healthy Men and the Influence of Food on Oral Pyridostigmine Pharmacokinetics"

The clinical portion of this study was initiated on March 11, 1990 and had been completed on May 26, 1990. The collection and analysis of the data continued in preparation for drafting the task report. The date the final report was issued is listed in Appendix A. Appendix J provides a summary of the results.

Appendix A

Task Order Final Reports: Dates of Issue

Task Order #7	August 7, 1993
Task Order #8	September 28, 1991
Task Order #9	September 29, 1991
Task Order #10	August 21, 1991
Task Order #11	November 11, 1991
Task Order #12	October 22, 1991
Task Order #13	April 18, 1991
Task Order #14	February 16, 1993

APPENDIX B

SUMMARY T.O. #7

Pyridostigmine bromide may be a useful adjunct to atropine sulfate to prevent death from organophosphate exposure if given in advance of the exposure and if given in a dose that is adequate to inhibit red blood cell acetylcholinesterase by 20-40%. The inter-individual variability to a known, constant exposure to pyridostigmine is poorly characterized.

This study was designed (1) to assess the relationship between plasma concentrations of pyridostigmine and erythrocyte acetylcholinesterase inhibition, (2) to determine whether erythrocyte acetylcholinesterase and the contractile response of the iris to light are inhibited in a parallel fashion by pyridostigmine, and (3) to assess the inter-individual variations in the concentration-effect relationships described in 1 and 2.

The clinical portion of the study was conducted between 27 July and 16 October 1987, and showed that the constant intravenous infusion of low doses of pyridostigmine was safe and well tolerated. Intravenous pyridostigmine gave relatively steady plasma concentrations of pyridostigmine and erythrocyte acetylcholinesterase inhibition during the latter portion of the infusions. Mean peak erythrocyte acetylcholinesterase inhibitions were 29% and 36% for infusions of 12.5 mcg/minute and 18.75 mcg/minute, respectively. The mean rate constant of elimination was 1.365 hr^{-1} , corresponding to an elimination half-life of 30 minutes. The mean plasma clearance was 44.62 L/hr, or 744 ml/minute. The mean concentration at which 50% of the erythrocyte acetylcholinesterase activity was inhibited (IC_{50}) was 31.8 ng/ml. The

influence of these infusions on the contractile response of the iris was difficult to measure and could not be reliably standardized due to technical limitations. Because these problems introduced serious uncertainty into measuring pupillary response to the infusions, no correlation with plasma pyridostigmine levels or acetylcholinesterase inhibition could be assessed.

APPENDIX C

SUMMARY T.O. #8

Pyridostigmine bromide may be a useful agent to prevent death from organophosphate exposure if given in advance of the exposure and if given in a dose that is adequate to inhibit red blood cell acetylcholinesterase by 20-40%. A previous study conducted at our institution determined that 16 mg of pyridostigmine bromide administered as syrup inhibited red blood cell acetylcholinesterase by 20-40% within one hour after dosing, but the inhibition fell below 20% by about five hours after dosing. In an effort to find a formulation that would provide adequate acetylcholinesterase inhibition for a longer period, sustained-release formulations are being developed.

This study was designed (1) to compare the pyridostigmine bromide pharmacokinetics in healthy men after single doses of 22 mg of pyridostigmine bromide as syrup and after 22 mg of pyridostigmine bromide in two different sustained-release formulations, (2) to characterize the time course of red blood cell acetylcholinesterase inhibition following the administration of the two dosage forms of oral sustained-release pyridostigmine bromide and compare these to the inhibition occurring after oral pyridostigmine bromide syrup, (3) to assess the pharmacodynamic relationship between pyridostigmine plasma levels and erythrocyte acetylcholinesterase inhibition, and (4) to assess the safety and tolerance of these two sustained-release formulations compared to pyridostigmine bromide syrup.

The clinical portion of the study was conducted between 11 January and 23 April 1988. The two sustained-release formulations were less well absorbed than the pyridostigmine syrup, measured either by direct plasma level assay or by effect on red blood cell acetylcholinesterase activity. The

relative bioavailability of the "slow-release" tablet compared to syrup was 51% by direct plasma level measurement and 60% by effect on acetylcholinesterase activity. The relative bioavailability of the "fast-release" tablet compared to syrup was greater than that for the slow-release tablet, 62% by direct plasma level measurement and 83% by effect on acetylcholinesterase activity. Doubling the dose of fast-release tablets still did not achieve the plasma levels or acetylcholinesterase inhibition of the syrup at half the dose. The subjects tolerated the administration of pyridostigmine well.

APPENDIX D

SUMMARY T.O. #9

Pyridostigmine bromide may be a useful agent to prevent death from organophosphate exposure if given in advance of the exposure and if given in a dose that is adequate to inhibit red blood cell acetylcholinesterase by 20-40%. A previous study conducted at our institution determined that 16 mg of pyridostigmine bromide administered as syrup inhibited red blood cell acetylcholinesterase by 20-40% within one hour after dosing, but the inhibition fell below 20% by about five hours after dosing. Another study showed that 22 mg of syrup gave an average peak inhibition of 35% and provided at least 20% inhibition for slightly over 4 hours. In an effort to find a formulation that would provide adequate acetylcholinesterase inhibition for a longer period and be more convenient for dosing than syrup, sustained-release formulations are being developed.

This study was designed (1) to compare the pyridostigmine bromide pharmacokinetics in healthy men after single doses of 45 mg of pyridostigmine bromide as syrup and after 45 mg of pyridostigmine bromide in two different sustained-release formulations, (2) to characterize the time course of red blood cell acetylcholinesterase inhibition following the administration of the two dosage forms of oral sustained-release pyridostigmine bromide and compare these to the inhibition occurring after oral pyridostigmine bromide syrup, and (3) to assess the safety and tolerance of these two sustained-release formulations compared to pyridostigmine bromide syrup.

The clinical portion of the study was conducted between 28 March and 30 April 1988. The two sustained-release formulations were less well absorbed than the pyridostigmine syrup, measured either by direct plasma level assay or by effect on red blood cell acetylcholinesterase activity. The relative bioavailability of the "slow-release" capsule compared to syrup was 41% by direct plasma measurement and 47% by effect on acetylcholinesterase activity. The relative bioavailability of the "fast-release" capsule compared to syrup was greater than that for the "slow-release" capsule, 70% by direct plasma level measurement and 77% by effect on acetylcholinesterase activity. The subjects tolerated the administration of pyridostigmine well.

- 767** Multiple-Dose Pharmacokinetics, Safety, and Tolerance of WR 6026, a Promising Drug for *Pneumocystis carinii* Pneumonia. **AG. PETTY***, **ON. KORNHAUSER**, **EG. PROBST**, **W. FLECKENSTEIN**, **PS. LIETMAN**. Johns Hopkins University, Baltimore, Maryland and Walter Reed Army Institute of Research, Washington, D.C.
WR 6026 (WR), an 8-aminoquinoline similar to primaquine, may be a useful agent to treat *Pneumocystis carinii* pneumonia. To find a dose that would provide desired WR plasma levels without unacceptable toxicity, a rising multiple-dose, randomized, double-blind, placebo (P)-controlled study was conducted.
WR was given once daily at 5, 15, 30, 45 or 60 mg/day for 14 days to healthy men. Clinical laboratory tests and symptoms were monitored. Blood samples were obtained for drug assay by HPLC to characterize pharmacokinetics.
There were no significant differences between the WR and P groups in the incidence or severity of laboratory abnormalities except for a slightly greater fall in the hematocrit of WR vs. P subjects (3.1% vs. 1.1%). Increasing dose of WR caused reversible, asymptomatic methemoglobinemia (M). All subjects at 60 mg/day had M, with peak M of 1.8-6.9% (normal 0-1.5%) occurring between days 8 and 17.
The mean peak plasma concentration was 110 ng/ml at 60 mg/day (range 66-210 ng/ml). The mean terminal elimination half-life was 29 hours. There was no evidence of saturation kinetics. These data indicate that WR may be dosed once daily.
- 768** Viral Ecology of Acute Diarrhea in Hospitalized Young Children. **PENELOPE H. DENNEHY***, **RODOLFO E. BEGUE**, **SARA SPANGENBERGER**, **BARBARA A. VELOUDIS** AND **GEORGES PETER**. Brown University and Rhode Island Hospital, Providence, RI.
To determine the role of enteric viruses in the ecology of acute community and hospital acquired diarrhea, children ≤ 2 yrs of age admitted with diarrhea and those developing diarrhea after 2 days of hospitalization had stool specimens collected within 48 hrs of admission or at onset of diarrhea. Stools were tested for rotavirus (RV) and enteric adenovirus (EAd) by commercial monoclonal antibody-based ELAs and for other viruses by direct EM and tissue culture. Diarrhea was defined as ≥ 2 loose or watery stools per day, duration ≤ 10 days and no other cause for these symptoms.
During the two years of this ongoing study to date 272 (94%) of 2834 patients admitted to the ward for children 0-2 yrs had diarrhea and were enrolled. Review of virology laboratory records for a one year period indicated that $\leq 5\%$ of patients with diarrhea were not enrolled. 182 (67%) patients had diarrhea on admission and 90 (33%) acquired disease during hospitalization. 19 (10%) with C/A diarrhea had bacterial diarrhea and in 65 (36%) enteric viruses were found. RV accounted for ~ 4 (70%) of the enteric viral infections, while EAd, astrovirus and coronavirus accounted for 11 (17%), 3 ($\leq 1\%$) and 2 ($\leq 1\%$) cases respectively. 5 dual infections occurred: 3 with RV/EAd and 1 each with RV/astrovirus and RV/salmonella. In contrast, of patients with H/A disease, some had bacterial diarrhea and 24 (26%) had enteric viruses. RV accounted for 20 (83%) infections and astrovirus 1 ($\leq 1\%$); 3 (1%) were dual infections with RV/EAd. No pathogens were isolated in 99 (34%) C/A and 66 (73%) H/A cases. Review of symptoms indicates that children admitted with diarrhea due to RV were significantly more likely to be dehydrated than children with other bacterial or viral agents ($p < .005$).
In summary, viral pathogens were found in 35% of young children with diarrhea. The ecology in the majority of cases, despite our techniques, remains undetermined.
- 769** Role of Isotype-Specific and VP7-Specific Serum Antibodies in Protection Against Natural Rotavirus Infections. **M.L. O'RYAN***, **D.O. MATSON**, **I. HERRERA**, **M.K. ESTES**, **L.K. PICKERING**. University of Texas Medical School and Baylor College of Medicine, Houston, TX.
Understanding the immunology of rotavirus infection is crucial for vaccine development. We sought to determine if serum anti-rotavirus isotype-specific and VP7-specific antibodies correlated with protection against natural rotavirus infections. The immune response following rotavirus infections was also characterized. 198 children attending 4 day care centers (DCC) in Houston were monitored during one rotavirus season (10/89-4/90) with weekly stool collections. 45 children had 2 serum samples drawn, each separated by at least 2 months. 80 rotavirus infections (as determined by stool shedding) occurred in 72 children; 63% were symptomatic; all but one were caused by serotype 1 virus. Children not infected with rotavirus ($n=21$) had higher serum anti-rotavirus IgA titers ($p=.02$), IgG titers ($p=.08$) and epitope blocking titers (EBT) to type 1 VP7 ($p=.01$) than children who became infected ($n=11$). Initial anti-rotavirus IgA titers $> 1/800$ were detected in 12/21 non-infected and in 1/11 infected children ($p=.01$). EBT to serotype 1 of $> 1:10$ were detected in 10/21 non-infected and in 0/9 serotype 1 infected children ($p=.01$). 10/11 infected children had seroconversions of IgA, IgG and serotype 1 EBT compared to 4/21, 5/21 and 2/21 non-infected children, respectively ($p < .002$ to $< .001$). These data show that serum IgA and homotypic EBT correlates with protection against natural rotavirus infection and that anti-rotavirus specific serum antibody responses are directly associated with virus excretion.
- 770** Fecal Antirotavirus IgA Responses in Symptomatic, Asymptomatic and Uninfected Children. **M.L. O'RYAN***, **I. HERRERA**, **D.O. MATSON**, **M.K. ESTES**, **L.K. PICKERING**. University of Texas Medical School and Baylor College of Medicine, Houston, TX.
Intestinal IgA responses occur in children after rotavirus infection. Whether intestinal IgA protects against infection or disease is uncertain. We compared fecal antirotavirus IgA titers (fIgA) among children with rotavirus diarrhea, with asymptomatic infection, or with no detected infection. Children ($n=196$) attending 4 day care centers in Houston were monitored for rotavirus infection and fIgA antibody development during one rotavirus season (10/89-4/90). Symptoms were recorded daily and stool specimens were collected weekly and assayed for rotavirus by ELISA. For 51 infected children, fIgA titers were determined by ELISA one week before and three weeks after infection. For 51 children not infected according to our surveillance techniques, fIgA titers were determined in two samples four weeks apart. 84% of the infected children and 17% of the non-excretors had a four-fold or greater rise in IgA titer ($p < .001$). Among the infected children, 68% of the symptomatic and 79% of the asymptomatic children responded ($p=.1$) and fIgA titers achieved after infection were similar ($p=.42$). All children who had undetectable antibody levels $< 1:50$ ($n=12$) became infected ($p < .001$), most of whom ($n=10$) were symptomatic. Among children with initial fIgA antibody, no titer distinguished those infected from non-infected ($p=.21$) or those with symptomatic from those with asymptomatic infection ($p=.51$). This study indicates that fIgA responses are a good measure of rotavirus infection; symptomatic and asymptomatic infections induce a similar rise in fIgA titers; children without detectable antibody are at a higher risk of infection and disease; but preceding titers do not clearly correlate with protection against infection or disease.
- 771** Anti-minovirus Activity of Soluble Intercellular Adhesion Molecule-1 in Cell Cultures and Organ Cultures of Human Respiratory Epithelium. **E. ARRUDA***, **C. CRUMP**, **S. MARLIN**, **V. MERLUZZI**, and **F. HAYDEN**. University of Virginia, Charlottesville, Va and Boehringer Ingelheim Pharmaceuticals, Ridgefield, Ct.
Intercellular adhesion molecule-1 (ICAM-1) is the cellular receptor for major-group minoviruses (RV). We have assessed the in vitro anti-minoviral activity of a soluble form of ICAM-1 (sICAM-1) in multiple-cycle, cytopathic effect (CPE)-inhibition assays. 1.0-3.2 $\mu\text{g/ml}$ sICAM-1 inhibited RV CPE in WI-38 fibroblasts and HeLa cell monolayers infected by 100 TCID₅₀ of 10 different major-group serotypes of minovirus. No direct inactivation of viral infectivity was found when RV-39 (10^6 or 10^7 TCID₅₀) was incubated with 32 $\mu\text{g/ml}$ sICAM-1 for 1 hour at 33°C. The effect of the timing of addition was studied in HeLa cells infected at MOI of 3 TCID₅₀/cell; virus yield at 10 hours was $\sim 10^6$ TCID₅₀/ml in controls. sICAM-1 10 $\mu\text{g/ml}$ completely inhibited replication when added 1 hour before or at the time of inoculation. No significant reductions ($\leq 10^{0.5}$) were observed with addition at 15, 30, 60, or 180 min. Monolayer exposure to sICAM-1 for 1 hour prior to inoculation followed by removal did not reduce yield, which suggested the lack of a residual effect. The ability of sICAM-1 to inhibit RV-39 infection of human respiratory epithelium was tested in adenoid explants exposed to 2000 TCID₅₀ RV-39. Supernatant titers at 24, 48 and 72 hours post-inoculation found complete inhibition of virus yield with sICAM-1 10 $\mu\text{g/ml}$ compared to controls ($\sim 10^6$ TCID₅₀). sICAM-1 was inhibitory to the replication of major-group minoviruses in two cell lines and in human respiratory epithelium. Its inhibitory effect was present early in the replicative cycle, but the lack of reduction in viral infectivity by direct incubation with sICAM-1, suggests that the virus-sICAM-1 interaction is readily reversible.
- 772** Antigenic and Genomic Analysis of Respiratory Syncytial Virus (RSV) Isolates from a Study of Nosocomial Infection. **GA Storch***, **CB Hall**, **LJ Anderson**, **CS Park**, **DE Dobner**. Washington Univ Sch Med, St. Louis, MO; Univ of Rochester Sch Med, Rochester, NY; CDC, Atlanta, GA.
Recently developed techniques for antigenic and genomic analysis permit the recognition of differences among strains of RSV that were previously thought to be identical. We have used monoclonal antibody (MCA) analysis and ribonuclease protection (RP) to characterize isolates recovered during previously reported studies of nosocomial RSV carried out during the 1974-75 and 1975-76 seasons. Of 53 isolates recovered during those studies, 51 were group A and 2 were group B. Antigenic analysis of 43 group A isolates using MCAs to the G glycoprotein classified 32 as subgroup A/4, 9 as A/2, and 1 as A/7. RP analysis of the G glycoprotein gene of 36 group A isolates revealed patterns similar to, but distinct from, the patterns of isolates from the mid-late 1980s. The subgroup A/4 isolates included 2 distinct genomic variants, with minor genomic variability present within each, as well as among the A/2 isolates. During both RSV seasons studied, strains from at least 2 subgroups were isolated from patients and/or staff with nosocomial infections. Clusters of particular strains could be traced. We conclude that some nosocomial outbreaks of RSV consist of multiple distinct episodes of transmission of different strains of RSV that are circulating concurrently.

APPENDIX F

SUMMARY T.O. #10

WR 6026, an 8-aminoquinoline similar to primaquine, may be a useful agent to treat visceral leishmaniasis and may hold an advantage over other compounds used currently (e.g., pentavalent antimony, pentamidine, and amphotericin B) in being more convenient and less toxic. A previous study conducted at our institution determined that a single dose of 60 mg of WR 6026 administered as four 15 mg capsules was well tolerated with no adverse symptoms and only minor elevations of serum aminotransferases, mild elevations in lactic dehydrogenase, and a single case of minimally elevated serum triglycerides. None of the subjects had an increase in methemoglobin. There was a lag time of about 30 minutes between drug administration and detectable plasma levels, a fourfold variation in areas under the plasma concentration-time curve, and a half-life of 5.2-17.3 hours (mean 10.7 hours). In an effort to find a dose that would provide desired WR 6026 levels in the blood over long periods without unacceptable toxicity, a rising multiple-dose study was conducted.

This study was a randomized, double-blind, placebo-controlled investigation designed (1) to determine the pharmacokinetics of WR 6026 at doses of 5 mg, 15 mg, 30 mg, 45 mg and 60 mg given once daily over 14 consecutive days in healthy men and (2) to assess the safety and tolerance of these doses.

The clinical portion of the study was conducted between 16 May and 16 December 1988. Four subjects (two drug, two placebo) were studied at 5 mg, 30 mg and 45 mg per day; eight subjects (four drug, four placebo) were studied at 15 mg per day; and twelve subjects (six drug, six placebo) were studied at 60

T.O. #10

mg per day. The doses were generally well tolerated. One subject at 15 mg per day developed elevated serum triglycerides after 7 days, and so the drug was stopped. Thirty hours after the last dose was administered, this subject appeared to have a grand mal seizure. In retrospect, the subject may have had seizures on occasion before entering the study. No other subjects at this dose or any of three higher doses had clinically significant adverse events when compared to subjects receiving placebo in this study. The most frequent symptomatic complaint was headache, present in four subjects receiving WR 6026 and three subjects receiving placebo. Serum transferase elevations during the study were noted in five subjects receiving WR 6026 and seven subjects receiving placebo. Some, usually minor, electrocardiographic changes developed during the study in 10 subjects in each arm. The two groups did differ in the mean fall in hematocrit observed during the study, 3.4% in subjects receiving WR 6026 and 1.8% in subjects receiving placebo. This difference was statistically significant but clinically insignificant. Methemoglobin elevations were noted in all six subjects receiving 60 mg per day (peak methemoglobin 3.2 - 6.9%), and in one of two subjects who received 30 and 45 mg per day (1.8% and 3.4%, respectively).

The pharmacokinetic analysis of the serial blood concentrations indicate that the drug accumulates over time. The mean peak and trough WR 6026 concentrations increased with increasing dose. Using a two-compartment analysis, the mean beta elimination half-life was found to be 28.79 hours (range 13.13 - 49.46 hours). Median areas under the WR 6026 concentration-time curve were proportional to the dose of WR 6026, although there was two- to threefold intersubject variation at each dose. Areas under the curve when normalized to dose were similar at all doses, indicating that WR 6026

disposition obeys linear pharmacokinetics over the dosage range of 5 to 60 mg daily.

Methemoglobin concentrations increased with increasing WR 6026 doses. Again, there were intersubject variations at each dose level. However, the maximum increment in methemoglobin was highly correlated with the WR 6026 AUC. No signs or symptoms resulting from methemoglobinemia were noted in any subject.

We conclude that WR 6026 given in doses of up to 60 mg daily for 14 consecutive days is well tolerated. Further trials in subjects infected with leishmania are indicated to assess the activity and efficacy of WR 6026.

APPENDIX G
SUMMARY T.O. #11

Pyridostigmine bromide may be a useful agent to prevent death from organophosphate exposure if given in advance of the exposure and if given in a dose that is adequate to inhibit red blood cell acetylcholinesterase by 20-40%. A previous study conducted at our institution determined that 16 mg of pyridostigmine bromide administered as syrup inhibited red blood cell acetylcholinesterase by an average of 20% just over one hour after dosing, but the average inhibition fell below 20% by about five hours after dosing. Another study showed that 22 mg of pyridostigmine bromide in syrup gave an average peak inhibition of 35% and provided at least 20% inhibition for slightly over 4 hours. A third study showed that 45 mg of pyridostigmine bromide in syrup produced inhibition above 20% for at least 4 hours in all eight subjects, with a mean duration of at least 20% inhibition of 5.99 hours. The sustained-release tablets tested at equivalent pyridostigmine doses in each of these studies were inferior in absorption and acetylcholinesterase inhibition compared to the syrup. In an effort to find a formulation that would provide adequate acetylcholinesterase inhibition for a longer period and be more convenient for dosing than syrup, other sustained-release formulations are being developed. The Osmec[®] (an osmotic-delivery module) seemed promising since in vitro it releases drugs (including pyridostigmine) into solution at a constant, zero-order rate. If pyridostigmine were absorbed at a constant fraction of the amount released, then constant pyridostigmine concentrations and acetylcholinesterase inhibition could be achieved.

This study was designed (1) to compare the pyridostigmine bromide pharmacokinetics in healthy men after single doses of 47 mg of pyridostigmine bromide as syrup and after 141 mg of pyridostigmine bromide in an osmotic-

T.O. #11

delivery module formulation (Osmet^R), (2) to characterize the relative time course of red blood cell acetylcholinesterase inhibition following the administration of these two dosage forms of oral pyridostigmine bromide, and (3) to assess the relative safety and tolerance of this sustained-release formulation compared to pyridostigmine bromide syrup.

The clinical portion of the study was conducted between 8 January and 3 February 1989. The plasma pyridostigmine levels and reduction in red blood cell acetylcholinesterase activity were much lower after dosing with the Osmet^R than with syrup. The mean peak plasma pyridostigmine level was 7.4 ng/ml with the Osmet^R and 22.6 ng/ml with the syrup. The mean peak acetylcholinesterase inhibition was 19% with the Osmet^R and 40% with the syrup. The relative bioavailability of the Osmet^R formulation compared to syrup was 17.9%. After the syrup dosage, three of the eight subjects developed gastrointestinal symptoms and another subject became lightheaded with a reduction of diastolic blood pressure. Two subjects complained of being sleepy 1-4 hours after the Osmet^R dose, another developed nausea and vomiting 39 hours after the Osmet^R dose, and another developed soft stools about 5 hours after the Osmet^R dose.

SUMMARY T.O. #12

This study was designed (1) to compare the pyridostigmine bromide pharmacokinetics in healthy men after single doses of 90 mg and 180 mg of pyridostigmine bromide as Mestinon Timespan[®] tablets, (2) to characterize the time course of red blood cell acetylcholinesterase inhibition following the administration of the two doses of oral sustained-release pyridostigmine bromide, and (3) to assess the safety and tolerance of these two doses of sustained-release pyridostigmine bromide.

The clinical portion of the study was conducted between 12 December and 23 December 1988, and showed that the two doses were well absorbed. One subject became lightheaded at approximately the same time as his erythrocyte acetylcholinesterase inhibition peaked at 58%. Another subject developed gastrointestinal discomfort about an hour after each dose, but whether this was due to missing breakfast rather than the drug is not clear, particularly since the symptoms were more severe with the lower dose. The mean peak inhibition for the four subjects receiving the 90 mg dose was 47% (range 39-58%), and the inhibition was 20% or greater for a mean of 6.81 hours (range 4.25-11.50). The peak inhibition for the two subjects who received the 180 mg dose was 62%, and the duration of inhibition of 20% or greater was 9.5-11.5 hours (mean 10.5).

APPENDIX I

SUMMARY T.O. #13

Niclosamide is a topical anti-schistosomal agent intended to prevent the penetration of schistosomal cercariae through the skin. To be effective it must be applied regularly in anticipation of exposure. Because of its structural similarity to known topical photoallergens, it may induce contact sensitization and/or contact photoallergy upon repeated application. To assess these possibilities, we conducted this study in a total of 45 healthy white subjects. Caucasian subjects were used to enhance our capacity to observe irritancy and allergic responses compared to more pigmented races (blacks or Orientals).

This study was conducted in two parts. The first part was designed to determine the irritancy threshold of niclosamide in petrolatum and of sodium lauryl sulfate (SLS). This portion of the study was conducted in 20 subjects as a randomized, double-blind, placebo-controlled investigation. The second portion of the study was designed to determine the contact sensitization and contact photoallergic potential of niclosamide and was conducted in 25 subjects. The contact sensitization portion was performed as an open study and was carried out in two parts -- an induction phase and a challenge phase.

The first portion of the study showed that the irritancy threshold for niclosamide in petrolatum was 75% (weight/weight). However, because this concentration was so dry and flaky, and therefore would not remain apposed to the skin in the contact sensitization phase, the next lowest concentration (50%) was used. The second portion of the study showed no evidence of contact sensitization or contact photoallergy in any of the 25 subjects upon rechallenge two weeks after completing the sensitization phase.

These results indicate a low likelihood of contact sensitization or contact photoallergy in response to topical niclosamide. We believe that with the benefit of these data, multiple-dose studies of both safety and efficacy can now be conducted.

APPENDIX J

SUMMARY T.O. #14

Pyridostigmine bromide may be a useful adjunct to atropine to prevent death from organophosphate exposure if given in advance of the exposure and if given in a dose that is adequate to inhibit red blood cell acetylcholinesterase by 20-40%. Previous studies conducted at our institution determined that single doses of pyridostigmine bromide administered as syrup inhibited red blood cell acetylcholinesterase by 20-40% for a longer period of time than equivalent doses of various "sustained-release" tablets and capsules; but syrup is inconvenient in a field situation. The best experimental sustained-released formulation (45 mg capsule) kept red blood cell acetylcholinesterase inhibition above 20% for four hours. In an effort to find a formulation that would provide adequate acetylcholinesterase inhibition for a longer period, other formulations are being investigated.

This study was designed (1) to characterize the pyridostigmine bromide pharmacokinetics in healthy men after single doses of two oral dosage forms of pyridostigmine bromide, one a sustained-release preparation and the other a standard tablet, and compare these to the pharmacokinetics of a prolonged intravenous pyridostigmine infusion, (2) to characterize the time course of red blood cell acetylcholinesterase inhibition following the administration of these two oral dosage forms and compare these to the inhibition occurring with intravenous pyridostigmine, (3) to assess the effect of food on the pharmacokinetics and pharmacodynamics of the two oral pyridostigmine dosage forms, and (4) to assess the safety, tolerance, pharmacokinetics, and pharmacodynamics of multiple doses of the oral formulations over two days.

The clinical portion of the study was conducted between 11 March and 26 May 1990, and showed that the mean bioavailability of the sustained-release tablet given fasting was 8% by pharmacokinetic assessment and 8% by assessment of the inhibition of erythrocyte acetylcholinesterase when compared to intravenous pyridostigmine. The bioavailability of the standard tablet given fasting was 17% by pharmacokinetic assessment and 19% by assessment of acetylcholinesterase inhibition. The effect of food on the inhibition of erythrocyte acetylcholinesterase activity was minimal for the sustained-release formulation, shifting the acetylcholinesterase inhibition-time curve about two hours to the right. On the other hand, food caused a reduction in the degree of acetylcholinesterase inhibition following the first standard tablet with food, but this effect was absent when the inhibition-time curve at steady state with feeding was compared to the fasting dose. Both formulations were well tolerated when given in multiple doses over two days.